No. 1040

Effects of Restriction of Salt Intake in Early Developmental Stage for the Organization of Neural Circuit for Gustatory Sense Processing

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Summary

The gustatory sense plays p pivotal role in the determination of palatability of potential food items. Received signals with the peripheral taste cells are transmitted to the gustatory neurons and finally conveyed to the brain via these neurons. For correct recognition of gustatory information, therefore, it is very important to wire gustatory nerve correctly within the developmental processes. Some previous studies indicated that restriction of dietary sodium during early embryonic period results in an enlarged gustatory terminal field in mammals. However, detail process and mechanisms of such abnormal neural development is unclear. To clarify these questions, it is critical to develop the methodology to visualize the structure of single or restricted number of gustatory neurons and observe them in living animal. This study tried to apply the IR-LEGO system, which utilizes infrared-laser irradiation to heat the cells and induction heat shock-mediated expression of transgenes in targeted single cells, for gustatory neurons of the zebrafish embryos or lavae to label them specifically by fluorescent proteins. Triple transgenic fish (Tg(*hsp70:gal4*)/Tg(*UAS:GFP*)/Tg(*HuC:mCherry*)) were used in this study. First, locations of RFP expressing gustatory ganglions (facial, grossopharyngeal, and vagal) were confirmed under the microscopic observation and suitable stage for heat shock to induce the transgene products (GFP) effectively were determined. According to these conditions, infrared-laser irradiated to neurons of each gustatory nerve ganglions in zebrafish embryos. As a result, GFP expression of ganglion neurons of facial and grossopharyngeal nerve were induced respectively. Co-expression of GFP both in grossopharyngeal and vagal ganglions were also observed. In these embryos, axons going to both central nervous system and peripheral area, and nerve terminal field in the brain were clearly visualized by GFP. This technique is very useful to observe the detailed structure of restricted number of gustatory neurons *in vivo* and to analyze the effects of restricted nutrition including sodium for establishment of normal gustatory nerve networks in detail.